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Influence of plankton concentration on gametogenesis and spawning of the black lip pearl oyster *Pinctada margaritifera* in Ahe atoll lagoon (Tuamotu archipelago, French polynesia)

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Abstract:

Pearl culture industry represents one of the dominant business sector of French Polynesia. However, it still entirely relies on unpredictable spat collection success. Our aim was to assess the influence of natural plankton concentration fluctuations on maturation and spawning of the black lip pearl oyster *Pinctada margaritifera*, during a 4 months survey conducted in Ahe atoll lagoon. Plankton concentration was assessed by chlorophyll *a* extraction and by microscope counts while gonadic index, gonado-visceral dry weights and histology were used to measure pearl oysters reproduction activity. We found that (i) plankton concentration fluctuations were mainly related to wind regime, (ii) gametogenesis rate was mainly related to plankton concentration, (iii) spawning occurred when maximal gonad storage was reached, (iv) plankton concentration was the main spawning synchronizing factor. These results contribute explaining *P. margaritifera* spat collection variability in French Polynesian atoll lagoon.

Highlights

► Reproduction determinism of *P. margaritifera* in an atoll lagoon of French Polynesia. ► We measured plankton composition and concentration. ► We measured gonadosomatic index of pearl oysters. ► Gametogenesis rate and spawning driven by plankton composition and concentration.

Keywords : Pearl Oyster ; Gametogenesis ; Spawning ; Phytoplankton ; French Polynesia

1. Introduction

Pearl culture industry represents one of the dominant business sector and source of income in French Polynesia. However, it still entirely relies on unpredictable natural reproduction and spat collection success. Indeed, large spatio-temporal variability of recruitment rates of the pearl oyster *Pinctada margaritifera* has been reported (Andréfouët et al. 2006, Thomas et al., this issue, a) and a better knowledge of the factors determining its reproduction is thus of particular interest.

Reproductive cycle of bivalve is generally driven by annual temperature cycles and by food availability (Bayne and Newell, 1983; Gervis and Sims, 1992; Sastry, 1979). Most studies on bivalve reproduction have been conducted in temperate coastal ecosystems which

are characterized by strong seasonal differences. Typically, temperature peaks in summer and concentration of phytoplankton is generally higher during spring, summer and early autumn than in late autumn and winter (Gómez and Gorsky, 2003; Valiela and Cebrián, 1999). In these environments, most bivalve species display an annual reproductive cycle characterized by a resting period during the coldest months of the year with spawning inhibited under a given temperature threshold (Dutertre et al., 2009). During spring and summer, the most favorable period for gametogenesis and spawning, food availability has a major impact on spawning frequency and reproduction effort (Enríquez-Díaz et al., 2008; Mac Donald and Thompson, 1985; Ruiz et al., 1992; Saucedo et al., 2002; Saxby, 2002). In addition to the seasonal determinism of gametes production and spawning, Bayne (1976) distinguished two types of energy management strategies used by bivalves to support gametogenesis. First, a "conservative strategy" where energy storage occurs prior to gametogenesis. Second, an "opportunistic strategy" where gametogenesis and storage can occur simultaneously.

However, both reproductive cycles and storage strategies are not species specific and a great plasticity has been observed between different populations of the same species. These differences were explained either by an adaptative physiological response to local environmental conditions or by genetic differences between populations (Hilbish and Zimmerman,1988; Loosanoff and Nomejko, 1951; Paulet et al., 1988; Thompson, 1984).

In tropical ecosystems, characterized by low seasonal variations and high water temperature, gametogenesis is generally continuous and spawning can occur all year long (Arjarasirikoon et al., 2004; Báqueiro-Cárdenas and Aldana-Aranda, 2000; Fournier, 1992; García-Domínguez et al., 1996; Gervis and Sims, 1992; Lefort and Clavier, 1994; Luna-González et al., 2000).

In the atoll lagoons of the Tuamotu archipelago (French Polynesia), temperatures are high and stable (between 25°C and 31°C) while plankton and particulate organic carbon concentrations are low with little seasonal differences but with high day to day fluctuations (Buestel and Pouvreau, 2000; Charpy et al., 1997; Thomas et al., 2010). In these lagoons, *P. margaritifera* has a continuous and fast gametogenesis leading to frequent asynchronous spawning all year long (Pouvreau et al., 2000). Pouvreau et al. (2000b) and Le Moullac et al. (2011) further demonstrated that *P. margaritifera* had low energy storage abilities and could therefore be defined as an "opportunistic" bivalve, investing all energy surplus into its reproduction. However, the precise influence of natural fluctuations of plankton composition and concentration on gametogenesis and spawning of pearl oysters remain poorly known.

In the present study, we aim to measure the influence of natural plankton concentration fluctuations

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on maturation and spawning of the black lip pearl oyster *P. margaritifera* during a four months survey conducted in Ahe atoll lagoon. To reach this goal, we used digitized images of visceral-mass sections (including gonads) to calculate a quantitative descriptor of gonadal maturity. We measured chlorophyll *a* concentration as a proxy for phytoplankton concentration and we estimated microplankton concentration by microscope counts of dinoflagellates, diatoms and ciliates.

MATERIALS AND MEHTODS

For convenience sake, on all graphs, time is represented in days. Day 1 represents the 7th of February 2009 and Day 120 represents the 6th of June 2009.

Study site

This study was conducted in Ahe atoll lagoon, located 500 km northeast of Tahiti island in the north of the Tuamotu archipelago (Figure 1). Ahe lagoon has an area of 142 km^2 and a mean depth close to 42 m with several maxima close to 70 m. Ahe is defined as a semi-enclosed atoll. There is one active pass in the west part of the lagoon and several reef-flat spillways (less than 50 cm depth) are distributed along the reef rim, mainly in the south and west parts of the lagoon. The average water renewal time (ratio of lagoon volume to average water input rate) was estimated at 34 days (Pagès and Andréfouët, 2001). Dumas et al. (this issue) recently characterized with numerical models the spatial variation of residence and flushing time in different weather conditions, and the average renewal time was estimated to be around 80 days.

To study the reproduction of *P. margaritifera*, 2000 6-years old pearl oysters were hung at low density (<20 pearl oysters m^{-3}) in December 2008 on a breeding line located in the north east of Ahe lagoon at approximately 3 km off the coast, at 10m deep (Figure 1). Experiments started in February 2009.

Meteorological and Hydrological parameters

Hourly wind direction and velocity were obtained from Takaroa atoll meteorological station (Météo France data) located about 120 km east of Ahe (145°3'4''W, 14°28'57''S). Daily mean of wind velocity was calculated from initial wind speed hourly values. Water temperature (°C) and salinity (PSU) were obtained from a Sea Bird (SBE V19 plus) probe immersed at a depth of 10 meters at the experimental breeding station (Figure 1).

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Plankton concentrations

Plankton concentration was measured at the breeding site every 3 days, between the 11th of February (Day 5) and the 2nd of June (Day 116). All sampling and analysis were done in triplicate. Water was sampled at 10m deep with a 5 liters Niskin bottle and gently transferred into 5 liters containers which were kept in the dark in an isotherm container and immediately brought back to the laboratory for analysis. Phytoplankton concentration was measured on all samples while microplankton enumeration was carried out on samples collected between the 11th of March (Day 33) and the 19th of May (Day 102) only.

Methods used to measure phytoplankton and microplankton concentration is described in details by Fournier et al. (this issue). Briefly, phytoplankton concentration was assessed by measuring chlorophyll *a* (Chl *a*) concentration for two size fractions : < 2 µm (Chl *a* < 2 µm) and > 2 µm (Chl *a* >2 µm). Water samples of 200 ml were filtrated sequentially on 2 µm Millipore filters and on GFF filters. Chl *a* concentration was measured with a Tuner Design TD 700 fluorometer equipped with the set of optical filters recommended by Welshmeyer (1994) for direct measurement of Chl *a*. Total phytoplankton (Chl *a* Tot.) concentration was defined as the sum of Chl *a* < 2 µm concentration and of Chl *a* >2 µm concentration.

To assess microplankton concentration, water samples (200ml) were fixed with alcalin lugol iodine. Enumeration of dinoflagellates, diatoms and dinoflagellates was carried out after sedimentation in Utermohl settling chambers (Hydro bios combined plate chamber), at 400 magnification with a Leica DMI 3000 inverted microscope and following the systematic literature (Kahl, 1931; Lee, 1985; Nezan, 1996; Paulmier, 1997; Ricard, 1987 and Sournia, 1986).

Dissection and dry flesh weight, gonadic index and maturity stages

Every 10 days, 80 pearl oysters were randomly collected from the breeding line between 18th of February 2009 (Day 12) and 5th of June 2009 (Day 119). After collection, pearl oysters were cleaned from epibionths and immediately brought back to laboratory. Dorso-ventral height and antero-posterior length (Gervis and Sims, 1992) were measured to the nearest mm with a soft stainless ruler. Mantle+gills, muscle and gonado-visceral mass were dissected, drained during 1h on absorbent paper and weighted to the nearest 0.01g. Wet weight of drained gonado-visceral mass (GVM), mantle+gills (MAN) and abductor muscle (MUS) were then converted into dry weights using their respective moisture content : 86 % for GVM, 87 % for MAN. and 75 % for MUS. These values come from average moisture content of 215 freeze dried pearl oysters sampled in Ahe lagoon, collected, dissected and drained as described above (unpublished personal data).

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From each set of 80 oysters, we measured the gonadic index of 40 randomly selected oysters and assessed their histological maturity stages. Specifically, gonado-visceral mass of pearl oysters were fixed during five days in a solution of 10% formalin prepared with seawater; transferred into 70% ethanol for preservation; cut in the sagittal plane and digitized with an Epson 2400 scanner. On the digitized images, gonad areas (GA, in pixels) and total gonado-visceral mass areas (GVMA, in pixels) were measured with the help of Image J freeware (http://rsbweb.nih.gov/ij/). Gonadic index (GI, in %) was then computed for each individual using the following equation : GI = GA / GVMA. Once digitized, gonado-visceral mass sampled between the 25th of March (Day 47) and the 9th of May (Day 92) were dehydrated through a graded ethanol series, embedded in paraffin, sectioned at 3–4 µm on a rotary microtome, stained with Giemsa dye and, finally, mounted on microscope slides. Sections were made in the gonad area, between the proximal end of the gut loop and the base of the foot.

Slide preparations were examined under a light microscope at 200x magnification to assess maturity stages, which were based on the description made by Pouvreau et al. (2000a) :

- Stage 0 : indeterminate or inactive, no evidence of gonadal development
- Stage 1 : Early gametogenesis, follicles small, gonia numerous
- Stage 2 : Actively developing but mature gametes are not observed
- Stage 3 : Near ripe follicles with mature gametes
- Stage 4 : Spawning ripe, follicles distended, confluent and entirely filled
- Rp : partially spawned, partially empty lumen
- Rt : spent, completely empty lumen

Data analysis, statistics

Mean of GI , of GVM dry weight, of MAN dry weight and of MUS dry weight were calculated for each sampling date. Since data were not normal, we used the non parametric Kruskal-Wallis test for the comparison of these four variables among sampling dates. *A posteriori* multiple comparisons were carried out using the non parametric Steel-Dwass test (Critchlow and Fligner, 1991; Spurrier, 2006).

As data were not normal, confidence intervals of GI , GVM dry weight, MAN dry weight and MUS dry weight were calculated using a boot strap method (Efron and Tibshirani,1986).

Spearman correlation analyzes were used to examine the relationships between wind velocity and concentration of Chl *a* < 2 µm, Chl *a* > 2 µm, Chl *a* Tot., dinoflagellates, diatoms and ciliates.

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Relationships between reproduction intensity and plankton concentration

To characterize the relationships between reproductive activity of pearl oysters and plankton concentration, we used a Spearman correlation analysis between the absolute variation of GI and the running mean of phytoplankton concentration.

The absolute variation of GI between two sampling dates was calculated using the following equation : $GIV = |GI_D - GI_{D-10}|$, where GIV = absolute gonadic index variation (%), GI = mean gonadic index of pearl oysters at the sampling date D (GI_D) and at the previous sampling date (10 days before $GI_D = GI_{D-10}$). Then, we calculated the running mean of phytoplankton concentration for six periods of time (5, 10, 15, 20, 25 and 30 days). Finally, we associated each GIV value with the values of running means calculated for the day corresponding to GI_D sampling date and we used a Spearman correlation analysis to test the relationships between GIV and the 6 running means of phytoplankton concentration.

We used the same procedure to test the relationships between phytoplankton concentration and variations of GVM, MAN and MUS dry weights.

In all tests, significance was determined with an alpha level of 0.05.

All analysis were conducted with the R freeware (http://www.r-project.org/).

RESULTS

Hydrobiological parameters

Oxygen concentration and salinity were stable during the period of our study (6.0±0.1 mg l^{-1} and 36.2±0.0 PSU, respectively). Water temperature ranged from 28.6°C to 29.2°C and maximum daily variation was 0.3°C.

Wind speed ranged from 0.7 m s^{-1} to 11 m s^{-1} (Figure 2a). When blowing from the east and south direction, wind velocity was higher than 6 m s^{-1} whereas it was lower than 6 m s^{-1} when blowing from west and north direction. Temperature, salinity, wind speed and wind direction corresponded to the usual climatic conditions expected during this period of the year (Buestel and Pouvreau, 1999; Thomas et al., 2010).

Chl *a* Tot. ranged from 0.22 µg l^{-1} to 0.60 µg l^{-1}. Mean concentration of Chl *a* < 2 µm (0.23 µg l^{-1}) was significantly higher than mean concentration of Chl *a* > 2 µm (0.14 µg l^{-1}) (Wilcoxon test, W = 157, p = 0.000). However, between Day 52 and Day 65, Chl *a* > 2 µm concentration was higher than Chl *a* < 2 µm concentration (Figure 2b).

The mean dinoflagellates concentration was 20.0±13.1 x 10^3 cell l^{-1}. The mean diatoms

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concentration was 7.3±12.2 x 10^3 cell l^{-1} and the mean concentration of ciliates was of 1.4±1 x 10^3 cell l^{-1}. Dinoflagellates constituted the dominant microplankton community (Figure 2c) except between Days 54 and 71 when diatoms concentration reached up to 6 x 10^5 cell l^{-1}.

All peaks of Chl *a* Tot. concentration occurred at the time of wind velocity peaks (Figure 2a, Days 15, 34, 57, 64,73, 99, 103 and 116). The lowest Chl *a* Tot. concentrations were measured during low wind periods (Figure 2a, Days 23, 78 to 92 and 109). Chl *a* Tot. concentration and wind velocity were significantly correlated (Table 1).

The four peaks of microplankton concentration were concurrent to Chl *a* > 2 µm concentration peaks (Figures 2b and 2c, Days 34, 57, 64 and 99). Similarly, the lowest microplankton concentration corresponded to the lowest Chl *a* > 2 µm concentration (Figures 2b and 2c, Days 73 to 92). Chl *a* > 2 µm and microplankton concentrations were also significantly correlated (Table 1).

GI and Maturity Stages

The fluctuations of the mean gonadic index (GI) and of the GI size class frequencies observed during our study are presented in Figure 2d. Mean GI displayed significant variations between sampling dates (Table 2).

From Day 12 to Day 47, the mean GI was at its highest and ranged from 0.24 to 0.29, with more than 70% of pearl oysters presenting a GI > 0.17. Between Day 47 and Day 64, a major spawning occurred. The mean GI decreased sharply from 0.29 down to 0.08 while the frequency of low GI (< 0.17) increased from 10% to 93%. Between Day 64 and Day 119, mean GI reached its lowest value (ca. 0.14) and low GI (< 0.17) frequency was high (70%).

The histological maturity stages also confirmed the main spawning event. Indeed, the frequency of ripe individuals decreased from 85% to 8% between Day 47 and Day 64 (Figure3).

During the study period, maturation was faster (between Day 12 and 20, Day 38 and 47, Day 64 and 73) and spawning was more intense (between Day 20 and 29, Day 47 and 55, Day 73 and 83) when the Chl *a* >2µm concentration was > 0.1 µg l^{-1}. Conversely, when Chl *a* > 2 µm concentration was < 0.1 µg l^{-1}, we only observed slight variations of mean GI (between day 12 and 20, day 38 and 47, day 64 and 73). These graphical observations were confirmed by a significant correlation between GIV and Chl *a* > 2 µm concentration running mean (Table 2).

Dry weights

Variation of mean gonado-visceral mass (GVM) dry weight, mean mantle+gills (MAN) dry weight

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and mean abductor muscle (MUS) dry weight are presented in Figure 2e. Mean GVM dry weight, MAN dry weight and MUS dry weight showed significant variations between sampling dates (Table 2).

Between Day 12 and Day 47 GVM dry weight was significantly higher than on Day 64. This confirmed the major spawning event observed during this period.

The MAN dry weight significantly increased when Chl *a* > 2 µm concentration was > 0.1µg l^{-1} (from Days 29 to 47 and Days 92 to 119) and significantly decreased during the main spawning event (between Day 47 and Day 74).

Compared to GVM and MAN dry weights, MUS dry weight was rather constant. We only observed a slight increase preceding the major spawning and a slight decrease after.

No significant relationships were reported between GVM dry weight and phytoplankton concentration (Table 3) neither between MUS or MAN dry weight and phytoplankton concentration (data not shown).

DISCUSSION

Hydrobiological parameters

We measured a Chl *a* Tot. mean concentration in the higher range of concentrations reported by Charpy and Blanchot (1998) and Pagès et al. (2001) in other French Polynesian atolls. The maximum concentration of Chl *a* Tot. observed during this study (0.6 µg l^{-1}) was close to values reported by Pagès et al. (2001) in Takaroa atoll lagoon during a phytoplankton bloom. Our mean concentration of ciliates and dinoflagellates were higher than those reported in Takapoto lagoon by Loret et al. (2000) but were in the range of measured in Tikehau lagoon (González et al., 1998).

Previous studies in French Polynesian atolls have shown that plankton concentration variations can be significant at small spatial and/or temporal scale, despite the average low concentrations and the weak seasonal differences (Buestel and Pouvreau, 2000; Charpy et al., this issue; Fournier et al, this issue; González et al., 1998; Pagano et al., this issue; Sournia et Ricard, 1976; Thomas et al., 2010). However, the exact mechanisms responsible for these changes remain unclear. Here, we report that the main fluctuations of Chl *a* > 2 µm, Chl *a* < 2 µm, diatoms and dinoflagellates concentration were clearly related to the wind regime variations.

The link between wind and plankton concentrations can be explained by a process of nutrient enrichment in the water column. First, in semi enclosed atoll lagoons, high winds (10 m s^{-1}) induce an overturning circulation that brings deep bottom water layer to the windward coast of the atoll

(Dumas et al., this issue; Lenhardt, 1991). This process brings nutrients accumulated in deep water layers to the surface layer. Indeed, nutrient release from the sediment added to remineralization of the settling organic particles tend to enrich bottom water layers of atoll lagoons (Charpy and Charpy-Roubaud, 1991; Charpy-Roubaud at al., 1996; Gerber and Marshall, 1982). Since experimental nitrogen and phosphorous enrichment of lagoonal water samples have increased growth and abundance of phytoplankton and heterotrophic flagellates (Ferrier-Pagès and Furla, 2001), this nutrient flow likely enhanced as well the plankton biomass and concentration in the eastern lagoon. Specifically here, south and east winds blowing at speed > 6 m s^{-1} probably brought enriched bottom water layer to the surface in the northeastern part of Ahe lagoon which promoted plankton growth and abundance where our breeding line was located. This process is indirectly confirmed by the low concentrations of Chl *a* Tot. observed during west and north winds < 6 m s^{-1}, and by results of Fournier et al. (this issue) who measured high Chl *a* Tot. concentration (> 1µg l^{-1}) in the northeastern part of the lagoon during steady east and south-east wind in October 2008.

Reproduction of pearl oysters

Dispersion of individual GI and histological results show that, during the period of our study, *P. margaritifera* exhibited a continuous reproductive activity with an extremely short resting period and a fast initiation of gametogenesis. These results are in agreement with previous studies which reported fast and continuous gametogenesis in tropical bivalves (Arjarasirikoon, 2004; Baqueiro-Cárdenas and Aldana-Aranda, 2000; García-Domínguez, 1996; Gervis and Sims, 1992).

Moreover, Ahe lagoon displays fairly similar hydrobiological conditions as Takapoto lagoon where Pouvreau et al. (2000b) showed that gametogenesis and spawning were occurring all year long. It is therefore obvious that *P. margaritifera* is characterized by continuous gametogenesis and spawning in Ahe atoll lagoon.

The effect of temperature, food availability and food quality on gametogenesis rate has mainly been studied for temperate species during experimental broodstock conditioning. These conditioning experiments demonstrated that an increase of temperature and food level were matched by an increase of maturation rate until an optimal combination of temperature and food was reached (Chávez-Villalba et al., 2002; Chávez-Villalba et al., 2003; Pronker et al., 2008; Martínez et al., 2003). From a bioenergetic point of view, these results are explained by the global increase of physiological rates when temperature increases and by the increase of energy inflow when food availability increases (Kooijman, 2000). Similar increase of gametogenesis rate with temperature and algae concentrations was observed in *P. margaritifera* conditioning experiments (personnal unpublished data).

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Once individuals are mature, a thermal stress is generally used to artificially induce spawning in hatcheries of temperate (Helm, 2004) and tropical bivalves (Gervis and Sims, 1992). However, in natural conditions factors inducing spawning remain unclear for temperate bivalves. A combination of several environmental factors have explained spawning, including thermal amplitude, phytoplankton blooms, tidal cycles and lunar phases (Bernard, 2011; Bonardelli et al., 1996; Starr et al., 1990).

Pouvreau et al. (2000b) and Le Moullac et al. (2011) have shown that gametogenesis and spawning of pearl oysters can occur all year long within a temperature range of 23°C to 31°C. Thus, in Tuamotu atoll lagoons, temperature is not a limiting factor for gametogenesis. The same authors have also concluded that sufficient plankton food is naturally available to sustain constant gametogenesis and spawning all year long. However, our results clearly demonstrate that gametogenesis rate and spawning of pearl oysters are directly related to plankton concentration.
In fact, conditions of food and temperature are met for *P. margaritifera* to produce gametes continuously all year long, but at a rate that varies with plankton concentration. Gametes accumulate in gonads until the maximum storage capacity is reached, which leads to spawning. Thus, when plankton concentration increases, gametogenesis rate increases, the maximum storage size of gonad is reached faster, and the number of individual spawning in the population increases as well.
Plankton concentration is therefore the main spawning synchronizing factor for pearl oysters in atoll lagoons. However, artificial spawning conducted at the Ifremer center of Vairao (Tahiti, French Polynesia) has revealed that female spawning was conditioned to the previous release of the male gametes (Le Moullac, pers com.). The impact of this gender synchronization is unknown *in situ* but is likely to play a role in the spawning synchronization of pearl oysters.

As discussed above, plankton concentration variations can be significant at small spatial and/or temporal scale. Thus, reproduction dynamics of pearl oysters is also likely to be highly variable from one site to another. A peak of plankton concentration at one site could induce a synchronized spawning of all individuals, while at other sites spawning may be reduced to a small percentage of individuals.
Seasonal variations of plankton concentration are commonly assumed to be low in Tuamotu atoll lagoons (e. g. Charpy, 1996). However, during the “warm” season (November to April), Buestel and Pouvreau et al. (2000b) and Thomas et al. (2010) measured higher concentration of phytoplankton than during the “fresh” season in Takapoto and Ahe lagoons, respectively. Available data are too scarce to demonstrate the impact of these seasonal variations on reproduction dynamics of pearl

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oysters. However, Pouvreau et al. (2000b) reported more intense spawning during the warm season than during the cool season and we also observed a major spawning at the end of the warm season.

To conclude, our results are in agreement with Pouvreau et al. 2000a who showed that *P. margaritifera* was an opportunistic species with very low energy storage abilities and which invest all surplus of energy into its reproduction. More specifically, our results clearly demonstrated that even if spawning can occur all year long, gametogenesis rate and spawning are tightly linked to the variation of food availability which itself is related wind regimes. Thus, spatial and/or temporal variability of the plankton concentration obviously leads to spatial and temporal heterogeneity of spawning intensity in the lagoon.

In association with the results of Thomas et al. (this issue a, b) who described the patterns of bivalve larval dispersal and growth in Ahe lagoon, our findings provide a comprehensive description of the processes involved in the inherent variability of spat collection success, observed empirically in Tuamotu atolls after decades of black pearl farming.

In fact, wind regime determines lagoon hydrodynamics regime which drives larval dispersal and impacts both food availability and reproduction dynamics. The monitoring of wind and of > 2µm plankton biomass is therefore a priority to predict spawning and infer subsequent larval dispersal (Thomas et al. this issue b).

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Table(s)

Table 1: Relationships between wind velocity (W.V.) and concentration of phytoplankton < 2µm (Chl *a* < 2 µm), phytoplankton > 2µm (Chl *a* > 2 µm), total phytoplankton (Chl *a* Tot.), dinoflagellates (Dino.), diatoms (Diato.), ciliates (Cili.) and total microplankton (MicPk); r = Spearman's rho, p = p-value. Significant correlations are indicated in bold type characters (α = 0.05).

	W.V.	Chl. *a* Tot.	Chl. *a* >2µm	Chl. *a* <2µm	Dino.	Diato.
Chl *a* Tot	**r = 0.56** **p = 0.000**					
Chl *a* >2µm	**r = 0.53** **p = 0.000**	-				
Chl *a* < 2µm	**r = 0.43** **p = 0.005**	-	**r = 0.45** **p = 0.004**			
Dino.	**r = 0.46** **p = 0.026**	**r = 0.54** **p = 0.007**	**r = 0.62** **p = 0.002**	r = 0.35 p = 0.103		
Diato.	**r = 0.49** **p = 0.018**	r = 0.21 p = 0.326	**r = 0.56** **p = 0.005**	r = -0.26 p = 0.224	r = 0.21 p = 0.335	
Cili.	r = 0.33 p = 0.129	r = 0.35 p = 0.106	r = 0.20 p = 0.371	r = 0.29 p = 0.185	r = 0.52 p = 0.118	**r = 0.11** **p = 0.000**
MicPk.	**r = 0.59** **p = 0.003**	**r = 0.56** **p = 0.006**	**r = 0.82** **p= 0.000**	r = 0.02 p = 0.910	-	-

Table 2 : Results of Kruskal-Wallis tests used for the comparisons of gonadic index (GI), gonado-visceral mass dry weight (GVM DW), mantle+gills dry weight (Ma. DW), muscle dry weight (Mu. DW) among sampling dates.

Test	df	Khi²	*p*
GI among sampling date	12	246	0.000
GVM DW among sampling date	12	172.6	0.000
Ma. DW among sampling date	12	52.7	0.000
Mu. DW among sampling date	12	23.3	0.025

Table 3 : Relationships between GIV (gonadic index variations) and running mean of phytoplankton concentration (phytoplankton < 2µm = Chl *a* < 2 µm, phytoplankton > 2µm = Chl *a* > 2 µm, total phytoplankton = Chl *a* Tot.) calculated for 6 different periods (5 to 30 days). Relationships between gonado-visceral mass dry weight variation and the same moving averages of phytoplankton concentration; r = Spearman's rho, p = p-value. Significant correlations are indicated in bold type characters (α = 0.05).

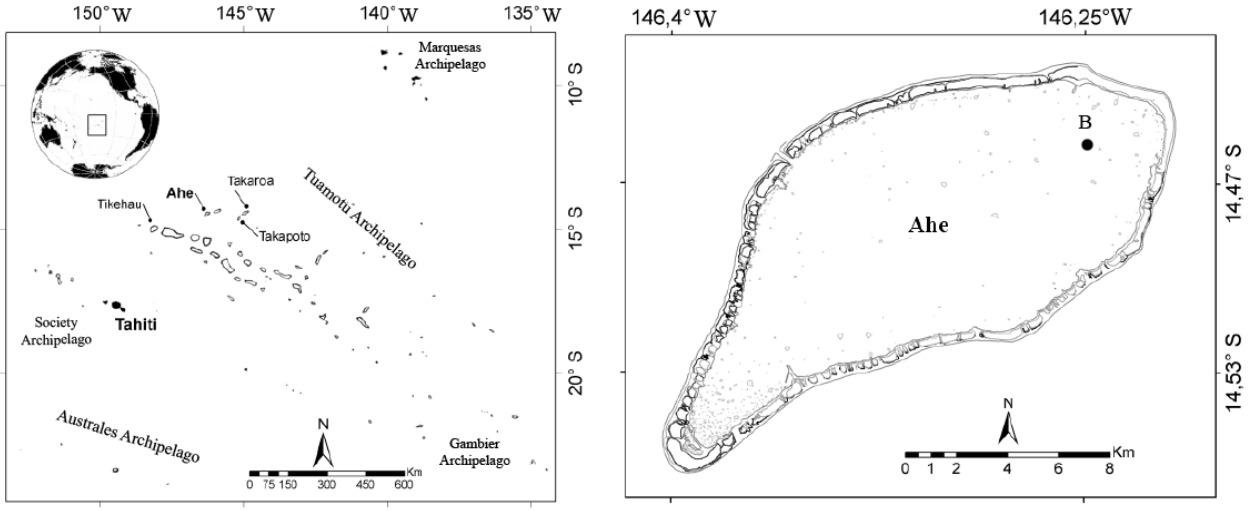
	GIV			Gonado-Visceral mass Variation		
Period (Days)	< 2µm	> 2µm	Tot	< 2µm	> 2µm	Tot
5	r = -0.45 p = 0.14	r = 0.20 p = 0.53	r = -0.20 p = 0.53	r = -0.15 p = 0.65	r = 0.43 p = 0.17	r = 0.15 p = 0.63
10	r = -0.36 p = 0.26	r = 0.49 p = 0.11	r = 0.15 p = 0.63	r = -0.01 p = 0.97	r = 0.4 p = 0.2	r = 0.4 p = 0.2
15	r = -0.32 p = 0.31	**r = 0.64** **p = 0.03**	r = 0.44 p = 0.15	r = -0.11 p = 0.73	r = 0.42 p = 0.17	r = 0.49 p = 0.11
20	r = -0.22 p = 0.50	**r = 0.59** **p = 0.04**	r = 0.43 p = 0.16	r = 0.10 p = 0.76	r = 0.18 p = 0.57	r = 0.38 p = 0.23
25	r = -0.34 p = 0.28	r = 0.39 p = 0.21	r = 0.29 p = 0.35	r = -0.04 p = 0.90	r = 0.13 p = 0.68	r = 0.18 p = 0.57
30	r = -0.27 p = 0.39	r = 0.34 p = 0.28	r = 0.31 p = 0.32	r = 0.06 p = 0.85	r = 0.10 p = 0.75	r = 0.22 p = 0.48

Figure 1 : Location of Ahe atoll and location of the experimental breeding station (B) in Ahe lagoon.

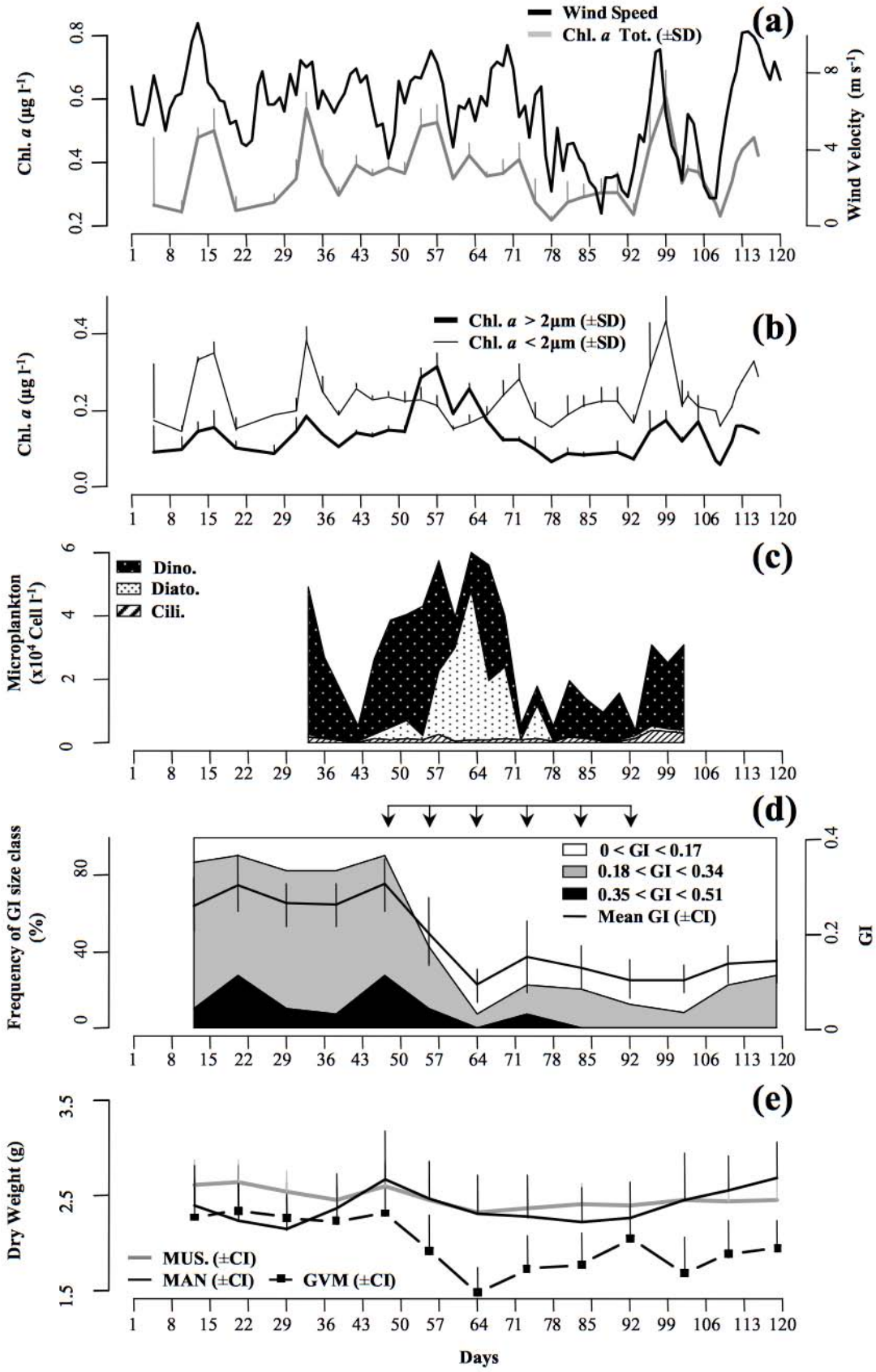
Figure 2 : (a) Wind velocity and total phytoplankton concentration (Chl *a* Tot.); (b) Chl *a* > 2µm and Chl *a* < 2µm concentration; (c) dinoflagellates (Din.), diatoms (Diat.) and ciliates (Cili.) concentrations; (d) mean gonad-index and frequency of 3 size class of gonadic index; (e) Dry weight of abductor muscle (MUS), of Mantle+Gills (MAN) and of gonado-visceral mass (GVM). All parameters were measured in Ahe atoll lagoon between the 7th of February 2009 (day 1) and the 6th of June 2009 (day 120). On figure 2d, arrows indicate the dates at which maturity stages of pearl oysters were assessed by histology.

Figure 3 : Frequency of maturity stages observed by histology between the 25th of March (day 47) and the 9th of May (day 92). E.D. = Early development (=Stage 1 + 2); M. / R. = Maturing + Ripe (=Stage 3 + 4) ; P.S. /S. = Partially spawn + Spent (=Stage Rp + Rt), U = Lack of gonadal tissue.

Figure(s)


150° W
145° W
140° W
135° W
10° S
15° S
20° S
Marquesas Archipelago
Takaroa
Ahe
Tuamotu Archipelago
Tikehau
Takapoto
Society Archipelago
Tahiti
Australes Archipelago
Gambier Archipelago
N
0 75 150 300 450 600 Km
146,4° W
146,25°W
14,47° S
14,53° S
B
Ahe
N
0 1 2 4 6 8 Km

Figure(s)


(a)
Wind Speed
Chl. a Tot. (±SD)
Chl. a (µg l-1)
Wind Velocity (m s-1)
(b)
Chl. a > 2µm (±SD)
Chl. a < 2µm (±SD)
Chl. a (µg l-1)
(c)
Dino.
Diato.
Cili.
Microplankton (x10^4 Cell l-1)
(d)
0 < GI < 0.17
0.18 < GI < 0.34
0.35 < GI < 0.51
Mean GI (±CI)
Frequency of GI size class (%)
GI
(e)
MUS. (±CI)
MAN (±CI)
GVM (±CI)
Dry Weight (g)
Days

Figure(s)